detector bound to the surface and molecules expressing the selected epitope in the sample.

REMARKS

Claims 1-14 are pending in the instant application. Claims 2-10 have been withdrawn from consideration by the Examiner and subsequently canceled by Applicants in this amendment. Claims 1 and 11-14 have been rejected. Claims 1 and 11 have been amended. No new matter has been added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. Finality of Restriction Requirement

The Examiner has made final the Restriction Requirement in the Office Action mailed February 27, 2002. Thus, in an earnest effort to advance the prosecution of this case, Applicants have canceled nonelected claims 2-10, without prejudice. However, in light of the finality of this Restriction Requirement, Applicants reserve the right to file a divisional application to the canceled subject matter.

II. New Matter in Claim 1

The Examiner suggests that language "contacting the amplified oligonucleotide with a fluorescent dye which binds to

RNA directly" which was added to claim 1 in the amendment filed by Applicants on June 19, 2002 is not supported by the specification. Applicants respectfully disagree.

In accordance with MPEP § 2163, there is no *in haec verba* requirement with respect to newly added claim limitations. Instead, claim limitations may be supported by express, implicit or inherent disclosure.

At page 11 of the specification, beginning at line 24, it is taught that:

> A preferred means for detection in the present invention comprises staining with a fluorescent dye. In this embodiment, after RNA amplification with a polymerase such as T7 RNA polymerase, T3 RNA polymerase, SP6 RNA polymerase or φ29 polymerase, a portion of the reaction mixture can be mixed with a fluorescent dye such as RiboGreen reagent (Molecular Probes, Inc) (U.S. Patent 5,436,134), a unsymmetrical cyanine dye that binds to RNA directly in the solution and then releases fluorescence signals. Examples of other fluorescent dyes with similar properties useful in this method include, but are not limited to, PicoGreen, TOTO-1 or YOYO-1.

Clearly, this teaching provides both express and implicit support for the amendment to claim 1 that a fluorescent dye which binds to RNA directly can be used. Thus, the amendment to claim 1 does not constitute new matter and properly overcomes the rejection under 35 U.S.C. § 102 set forth in the previous Office Action.

In the Advisory Action mailed January 29, 2003, the Examiner suggests that cited teachings at page 11, line 24 do not address claim language that the amplified oligonucleotide is amplified by RNA amplification. Applicants respectfully disagree since RNA amplification is explicitly stated at page 11, line 26, in a sentence which extends from page 11, line 25 to line 33. RNA amplification of the oligonucleotide is also explicitly taught in the application as-filed in Example 5 beginning at page 21. Further, in example 5, following RNA amplification, it is taught that the RNA product was contacted with the fluorescent dye RiboGreen reagent, which as taught at page 11, is a fluorescent dye which binds to RNA (see page 22, lines 6-14 of example 5). These teachings of the specification clearly convey to one skilled in the art that at the time the application was filed, applicants had possession of an invention wherein the oligonucleotide of an epitope detection was amplified by RNA amplification and the amplified oligonucleotide was contacted with a fluorescent dye with binds to RNA and stains the amplified oligonucleotide.

Withdrawal of this rejection under 35 U.S.C. §112, first paragraph is therefore respectfully requested.

III. Rejection of Claims 11-14 under 35 U.S.C. § 103(a)

The rejection of claim 11-14 under 35 U.S.C. § 103 over Suzuki et al. (Jpn. J. Cancer Res. Vol. 86 pg 885-889) in view of Eberwine et al. (U.S. Patent 5,922,553) and Zeytinoglu et al. (U.S. Patent 5,874,226) has been maintained. Specifically, the Examiner suggests that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Suzuki et al. by adding an additional step which is a transcriptase based reaction as taught by Eberwine et al. and Zeytinoglu et al. Arguments presented by Applicants that additional steps are not taught by these references were found unconvincing as the Examiner suggests that Zeytinoglu et al. disclose *in situ* immunodetection of antigens involving polymerase chain reaction (see col. 1, lines 36-54), the applicability of other amplification technologies including two steps (col. 5, lines 14-16) and the advantages of PCR amplification when the amount of antigen detected is very small (col. 5, lines 47-49). Further, the Examiner suggests that one of ordinary skill in the art would have been motivated to add more steps, either a reverse transcriptase reaction or a replicase based reaction with a reasonable expectation of

success.

Applicants respectfully traverse this rejection.

In the method of the present invention, the oligonucleotide of the epitope detector is amplified preferably via RNA amplification and the amplified RNA product is detected by contacting the amplified oligonucleotide of the epitope detector with a fluorescent dye or probe which binds RNA and stains the amplified oligonucleotide. In an earnest effort to advance the prosecution of this case, Applicants have amended claim 11 to clarify these distinguishing features. Support for the amendments to claim 11 can be found throughout the specification, for example at page 9, lines 1-7, page 9, line 33, through page 10, line 5, page 11, beginning at line 24, page 12, lines 20-27, Example 5 at pages 21-22 and claim 12, now canceled. Thus, no new matter is added by these amendments.

In contrast, the methods of both Suzuki et al. and Zeytinoglu et al. are related to DNA detection and not RNA amplification. As taught in Figure 1, at page 887 of Suzuki et al., the fluorescent compound ethidium bromide was used to locate DNA fragments in gel electrophoresis, and for staining the bound biotinylated DNA. Amplification techniques taught by Zeytinoglu et al. at column 5 also relate to DNA. Further, as acknowledged

by the Examiner, Zeytinoglu et al. teaches an *in situ* method wherein antibody-antigen contact occurs *in situ*. In contrast, the claims of the instant application are drawn to a methods wherein the antigen is extracted and immobilized to a surface. See steps (a) and (b) of claim 11. Thus, in the instant invention, contact between the antigen and epitope detector occurs *ex situ*. Accordingly, even were one of skill to include additional steps of Zeytinoglu et al. as suggested by the Examiner in the method of Suzuki et al., they would not arrive at the instant claimed invention for detection of an RNA product *ex situ*.

The reference by Eberwine et al. fails to remedy the deficiencies in the teachings of Suzuki et al. and Zeytinoglu et al. This reference does not teach use of epitope detectors comprising an oligonucleotide attached to a single chain Fv for the epitope or a constrained epitope specific CDR. Nor does this reference teach use of fluorescent dyes or probes which bind RNA and stain the amplified oligonucleotide.

Accordingly, since the combination of cited prior art references does not teach or suggest limitations in the claims relating to *ex situ* detection of an RNA product with fluorescent

dyes or probes, the cited prior art combination cannot render obvious the instant claimed invention.

Withdrawal of this rejection under 35 U.S.C. § 103 is therefore respectfully requested.

IV. Rejection of Claim 1 under 35 U.S.C. § 112, first paragraph

Claim 1 has been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention since the language "contacting the amplified oligonucleotide with a fluorescent dye which binds to RNA directly" is not supported in the specification.

Applicants respectfully traverse this rejection.

As discussed in Section II, *supra*, contrary to the Examiner's suggestion, the language "contacting the amplified oligonucleotide with a fluorescent dye which binds RNA directly" is both expressly and implicitly taught in the paragraph beginning at page 11, line 24. As taught therein, in a preferred embodiment of the invention the means for detection comprises staining with a fluorescent dye such as RiboGreen reagent

(Molecular Probes, Inc) (U.S. Patent 5,436,134), a unsymmetrical cyanine dye that binds to RNA directly in the solution and then releases fluorescence signals. Examples of other fluorescent dyes with similar properties to RiboGreen which are useful in the method of the present invention are also taught in this paragraph. This teaching in the specification describes the claimed invention in sufficient detail so that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention, thus meeting the written description requirements of 35 U.S.C. § 112, first paragraph. See MPEP § 2163 and Vas-Cath, inc. v. Mahurkar, 935 F.2d at 1563, 19 USPQ2d at 1116.

Withdrawal of this rejection under 35 U.S.C. § 112, first paragraph, is therefore respectfully requested.

V. Rejection of Claim 1 under 35 U.S.C. § 112, second paragraph

Claim 1 has been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner suggests that claim 1 is vague and indefinite because it is unclear what is meant by the language "directly" which describes the binding

of the RNA to the amplified oligonucleotide.

Applicants respect traverse this rejection.

At the outset, Applicants would like to clarify that contrary to the Examiner's suggestion "directly" does not describe the binding of the RNA to the amplified oligonucleotide, but rather describes a property of the fluorescent dyes used in the present invention. As taught at page 11 of the specification, preferred fluorescent dyes used in the present invention bind to RNA directly in solution and then release their fluorescence signal. Thus, what is meant by the term "directly" in claim 1, is clear when read in light of the content of the particular application disclosure as required by MPEP §2173.02.

However, in an earnest effort to advance the prosecution of this case, Applicants have amended claim 1 to remove this term and to clarify that the oligonucleotide of the epitope detector is amplified by RNA amplification and then contacted with a fluorescent dye which binds to RNA and stains the amplified oligonucleotide. Support for these amendments can be found in the specification at page 9, lines 1-7, page 9, line 33, through page 10, line 5, page 11, beginning at line 24, page 12, lines 20-27 and Example 5 at pages 21-22. Thus, no new matter is added by this amendment.

Withdrawal of this rejection under 35 U.S.C. § 112, second paragraph is respectfully requested in light of the amendments to the claims and the above remarks.

VI. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Pages have been attached hereto which show the changes made to the claims and specification and are labeled "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

Respectfully submitted,

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Date: **March 21, 2003**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel claims 2-10 and 12 without prejudice.

Please amend the claims as follows:

1. (amended) A method for quantifying molecules expressing a selected epitope in a sample comprising:

(a) immobilizing a molecule expressing a selected epitope in a sample to a selected surface;

(b) contacting the surface with an epitope detector so that the epitope detector binds to immobilized molecules on the surface, said epitope detector comprising an oligonucleotide attached to a monoclonal antibody for the selected epitope, a single chain Fv for the epitope or a constrained epitope specific CDR;

(c) amplifying the oligonucleotide of said epitope detector by RNA amplification;

(d) contacting the amplified oligonucleotide with a fluorescent dye which binds to RNA ~~directly~~ and stains the amplified oligonucleotide; and

(e) measuring fluorescence emitted from the stained oligonucleotide which is indicative of epitope detector bound to

the surface and molecules expressing the selected epitope in the sample.

11. (amended) A method for detecting molecules expressing a selected epitope in a sample comprising:

(a) immobilizing a molecule expressing a selected epitope in a sample to a selected surface;

(b) contacting the surface with an epitope detector so that the epitope detector binds to immobilized molecules on the surface, said epitope detector comprising an oligonucleotide attached to a monoclonal antibody for the selected epitope, a single chain Fv for the epitope or a constrained epitope specific CDR;

(c) amplifying the oligonucleotide of said epitope detector by RNA amplification;

(d) adding the amplified oligonucleotide of said epitope detector from step (c) to a reverse transcriptase based reaction or a replicase based reaction to increase sensitivity;

(e) detecting the amplified oligonucleotide of said epitope detector from step (c) by contacting the amplified oligonucleotide of said epitope detector from step (c) with a fluorescent dye or probe which binds RNA and stains the amplified oligonucleotide and measuring fluorescence emitted from the

stained amplified oligonucleotide which is indicative of epitope detector bound to the surface and molecules expressing the selected epitope in the sample.